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Geographic Spread of Epidemic Multiresistant *Staphylococcus aureus* Clone in Brazil

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Staphylococcus aureus **isolates from five large teaching hospitals and one medium-size community hospital located in geographically distant parts of Brazil, in the south and southeast (Rio de Janeiro, Niteroi, Sao Paulo, Porto Alegre) and in the north (Manaus), were tested for their antibiotic resistance patterns and genetic backgrounds. Eighty-five of the 152 isolates were identified as methicillin-resistant *S. aureus* (MRSA) by using a combination of an agar dilution screen and a *mecA* gene-specific DNA probe. All MRSA isolates were resistant to penicillin, erythromycin, gentamicin, oxacillin, and cephalothin, and the majority of isolates (74%) were also resistant to chloramphenicol, sulfamethoxazole-trimethoprim, ciprofloxacin, and clindamycin as well and were susceptible only to vancomycin. Isolates obtained from hospitals in Sao Paulo, Rio de Janeiro, Niteroi, and Porto Alegre (1,600 km from one another) and Manaus (3,700 km from Rio de Janeiro) were examined by a variety of molecular fingerprinting techniques: the nature of the *mecA* polymorph and Tn*554* attachment sites and restriction fragment length polymorphism of genomic DNAs after *Sma*I restriction and separation of the digested DNA by pulsed-field gel electrophoresis. The overwhelming majority of the isolates shared a common pulsed-field gel electrophoresis pattern and carried *mecA* polymorph III in combination with Tn*554* pattern B, indicating the presence of a single, epidemic MRSA clone spread over large geographic distances of Brazil.**

Application of molecular techniques has allowed the identification of several clones of methicillin-resistant *Staphylococcus aureus* (MRSA) which were responsible for disproportionately large fractions of disease in outbreaks in hospitals in various countries (1–3, 5, 10). In the studies presented here we used these techniques to compare MRSA isolates originating in six major Brazilian hospitals, located great distances from one another, either in the south and southeastern region or in the northern region of the country.

MATERIALS AND METHODS

Strains. One hundred fifty-two clinical *S. aureus* isolates were obtained during 1992 to 1994 from sources located in the south and southeastern regions of and from the northern region of Brazil. The hospitals and the MRSA isolates obtained from the particular hospital are listed in Table 1.

Agar screens. Agar screens with high bacterial inocula (100 μl of aerated overnight cultures grown in Trypticase soy broth at 37°C, representing 10^8 to 10^9 CFU) were plated onto Trypticase soy agar plates containing 25 μg of methicillin per ml by a previously described method (4, 12).

DNA probes. The DNA probe used was a *Pst*I-*Xba*I fragment of the *mecA* gene cloned into pTZ219 (11). A 5.5-kb *Eco*RV fragment was obtained from the plasmid containing Tn*554* (7, 9).

Determination of *mecA* polymorphs and Tn*554* patterns. Chromosomal DNAs were digested with the restriction endonuclease *Cla*I and were hybridized with the *mecA* probe. After removal of the probe the same gels were rehybridized with a Tn*554*-specific probe. The *Cla*I polymorphs and Tn*554* patterns were identified by comparison with previously described types (3, 5, 9). The preparation of the DNA and the nick-translation procedure to obtain the ^{32}P-labelled radioactive probe were carried out as described before (13).

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PFGE. Preparation of cells and fragmentation of their genomic DNA with *Sma*I was performed as described previously (3). Pulsed-field gel electrophoresis (PFGE) was performed as described previously (3) with the following pulse programs: 15 h with 20-s pulses, 7 h with 35-s pulses, 15 h with 50-s pulses, and 3 h with 90-s pulses. Some gels were run in a CHEF DR II apparatus (Bio-Rad, Richmond, Calif.) for 23 h at 14°C. The running conditions were as follows: the voltage was set at 200 V, ramped with an initial forward time of 1 s and a final forward time 30 s. Standard methodologies were used for staining, photographing, Southern hybridization, and probing of the gels (13).

Strains that shared PFGE patterns that differed from one another by fewer than four bands were assigned a common capital letter, with numerical indices representing subtypes, as described previously (4, 8, 14).

Methicillin resistance phenotype. The methicillin resistance phenotype was determined quantitatively through population analysis (4, 15).

RESULTS AND DISCUSSION

Major multiresistant epidemic MRSA clone in Brazil. In sharp contrast to the truly susceptible and borderline resistant strains, more than 70% of all MRSA isolates carried traits of resistance to at least nine different antibiotics (Table 2). All of the 85 MRSA isolates were also tested for their methicillin resistance phenotypes by a more quantitative method. All isolates showed an identical, class 3 phenotype (15).

All of the MRSA isolates from Rio de Janeiro (Hospital Universitario and Hospital Samaritano), Niteroi (Hospital Universitario), Porto Alegre (Hospital de Clinicas), Sao Paulo (Hospital dos Servidores do Estado), and Manaus (Hospital Universitario) were also characterized by molecular epidemiological typing methods, including PFGE, after digestion of chromosomal DNA with *Sma*I. While 21 different PFGE patterns were represented among the 85 MRSA isolates, the overwhelming majority of the isolates (66 of 85 [77%]) shared minor variants of a common PFGE pattern, referred to here as

TABLE 1. Origins and microbiological properties of Brazilian MRSA isolates

Strain	Town/hospital[a]	Yr of isolation	Infection or body site of infection[b]	Methicillin agar[c]	PAP[d]	Remaining antibiotic susceptibility[e]	*mecA* polymorph[f]	Tn*554* pattern	PFGE pattern[g]
BMB8592	Porto Alegre/HCPA	1992	Inv	CG	III	Vc, Cl	ND[h]	ND	A_2
BMB8692	Porto Alegre/HCPA	1992	Inv	CG	III	Vc	III	B	A_3
BMB9092	Porto Alegre/HCPA	1992	Inv	CG	III	Vc	III	B	E
BMB9892	Porto Alegre/HCPA	1992	Inv	CG	III	Vc, Cip	III	B	A_2
M7092	Manaus/HUFM	1992	Inv	CG	III	ND	III	ND	A_7
M7292	Manaus/HUFM	1992	Inv	CG	III	ND	ND	ND	A_1
M7392	Manaus/HUFM	1992	Inv	CG	III	ND	ND	ND	A_8
M7592	Manaus/HUFM	1992	Inv	CG	III	ND	III	ND	A_1
M7692	Manaus/HUFM	1992	Inv	CG	III	ND	ND	ND	A_1
M6894	Manaus/HUFM	1994	Inv	CG	III	ND	III	ND	A_8
M7194	Manaus/HUFM	1994	Inv	CG	III	ND	III	ND	A_8
M7494	Manaus/HUFM	1994	Inv	CG	III	ND	III	ND	A_7
BMB4192	Rio de Janeiro/HUCFF	1992	Inv	CG	III	Vc	III	B	A_4
HU12	Rio de Janeiro/HUCFF	1993	Bronchial washing	CG	III	Vc, Cip	ND	B	H_1
HU20	Rio de Janeiro/HUCFF	1993	Catheter tip	CG	III	Vc, SfT	II	ND	B
HU21	Rio de Janeiro/HUCFF	1993	Inguinal purulent drainage	CG	III	Vc, Cip, SfT	II	ND	B
HU22	Rio de Janeiro/HUCFF	1993	Blood	CG	III	Vc, Cip	ND	ND	H_1
HU23	Rio de Janeiro/HUCFF	1993	C	CG	III	Vc, Cip	III	ND	A_5
HU24	Rio de Janeiro/HUCFF	1993	C	CG	III	Vc, Cip	III	B	A_4
HU25	Rio de Janeiro/HUCFF	1993	C	CG	III	Vc, Co	III	B	A_1
HU27	Rio de Janeiro/HUCFF	1993	Transtracheal aspirate	CG	III	Vc	III	ND	A_4
HU28	Rio de Janeiro/HUCFF	1993	Blood	CG	III	Vc	III	ND	A_1
HU29	Rio de Janeiro/HUCFF	1993	C	CG	III	Vc	III	B	A_1
HU30	Rio de Janeiro/HUCFF	1993	C	CG	III	Vc	ND	B	H_1
HU34	Rio de Janeiro/HUCFF	1993	Purulent drainage	CG	III	Vc, SfT	II	ND	B
HU35	Rio de Janeiro/HUCFF	1993	Tumor fragment	CG	III	Vc, Cl	ND	ND	H_1
HU36B	Rio de Janeiro/HUCFF	1993	Skin wound	CG	III	Vc	III	ND	A_1
HU37	Rio de Janeiro/HUCFF	1993	Peritoneal secretion	CG	III	Vc, Cl	I	ND	C
HU39	Rio de Janeiro/HUCFF	1993	C	CG	III	Vc, Cl	III	ND	A_5
HU40	Rio de Janeiro/HUCFF	1993	C	CG	III	Vc	III	B	A_4
HU41	Rio de Janeiro/HUCFF	1993	C	CG	III	Vc	III	B	A_{12}
HU42	Rio de Janeiro/HUCFF	1993	C	CG	III	Vc	III	B	A_{13}
HU43	Rio de Janeiro/HUCFF	1993	C	CG	III	Vc	III	ND	A_1
HU44	Rio de Janeiro/HUCFF	1993	C	CG	III	Vc, Cip	III	B	H_2
HU46	Rio de Janeiro/HUCFF	1993	Transtracheal aspirate	CG	III	Vc	III	ND	A_4
HU47	Rio de Janeiro/HUCFF	1993	Surgical wound	CG	III	Vc	III	ND	A_4
HU53	Rio de Janeiro/HUCFF	1993	Transtracheal aspirate	CG	III	Vc, Cl	III	ND	A_5
HU60	Rio de Janeiro/HUCFF	1993	Surgical wound	CG	III	Vc	III	ND	A_1
HU62	Rio de Janeiro/HUCFF	1993	Ascites	CG	III	Vc	III	ND	A_1
HU72	Rio de Janeiro/HUCFF	1993	Urine	CG	III	Vc	III	B	A_4
HU73	Rio de Janeiro/HUCFF	1993	Catheter tip	CG	III	Vc	III	B	A_1
HU76	Rio de Janeiro/HUCFF	1993	Blood	CG	III	Vc	ND	ND	A_1
HO193	Rio de Janeiro/HS	1993	Bronchial washing	CG	III	Vc	III	B	A_1
HO293	Rio de Janeiro/HS	1993	C	CG	III	Vc	III	B	A_{10}
HO393	Rio de Janeiro/HS	1993	Transtracheal aspirate	CG	III	Vc	III	ND	A_1
HO493	Rio de Janeiro/HS	1993	Osteomyelitis	CG	III	Vc	ND	B	I
HO593	Rio de Janeiro/HS	1993	Blood	CG	III	Vc	III	B	A_1
H1493d	Rio de Janeiro/HS	1993	HCP	CG	III	Vc	III	ND	A_1
H1593	Rio de Janeiro/HS	1993	HCP	CG	III	Vc	III	ND	A_9
H4194	Rio de Janeiro/HS	1994	Transtracheal aspirate	CG	III	Vc	III	ND	A_1
H4494a	Rio de Janeiro/HS	1994	C	CG	III	Vc	III	ND	A_1
H4494b	Rio de Janeiro/HS	1994	C	CG	III	Vc	III	B	A_1
H4594	Rio de Janeiro/HS	1994	Cateter tip	CG	III	Vc	III	B	A_1
H4694	Rio de Janeiro/HS	1994	Transtracheal aspirate	CG	III	Vc	III	B	A_{11}
H5894d	Rio de Janeiro/HS	1994	C	CG	III	Vc	III	ND	A_1
H5894	Rio de Janeiro/HS	1994	C	CG	III	Vc	III	B	A_1
H5994	Rio de Janeiro/HS	1994	C	CG	III	Vc	III	B	A_1
H6064	Rio de Janeiro/HS	1994	Inv	CG	III	Vc	III	ND	A_1
H6194	Rio de Janeiro/HS	1994	Transtracheal aspirate	CG	III	Vc	III	B	A_1
H6294	Rio de Janeiro/HS	1994	Transtracheal aspirate	CG	III	Vc	III	B	A_1
BMB4792	Niteroi/HUAP	1992	Inv	CG	III	Vc	III	B	A_1
BMB5292	Niteroi/HUAP	1992	Inv	CG	III	Vc	III	B	A_1
BMB5592	Niteroi/HUAP	1992	Inv	CG	III	Vc	III	B	A_1
BMB5692	Niteroi/HUAP	1992	Inv	CG	III	Vc, SfT	III	ND	A_1
BMB5892	Niteroi/HUAP	1992	Inv	CG	III	Vc, Co	III	B	A_1
BMB5992	Niteroi/HUAP	1992	Inv	CG	III	Vc	III	B	A_1

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TABLE 1—*Continued*

Strain	Town/hospital[a]	Yr of isolation	Infection or body site of infection[b]	Methicillin agar[c]	PAP[d]	Remaining antibiotic susceptibility[e]	*mecA* polymorph[f]	Tn*554* pattern	PFGE pattern[g]
BMB6192	Niteroi/HUAP	1992	Inv	CG	III	Vc, SfT	ND	ND	B
BMB6492	Niteroi/HUAP	1992	Inv	CG	III	Vc	III	B	A_1
BMB6592	Niteroi/HUAP	1992	Inv	CG	III	Vc	III	B	A_1
BMB6692	Niteroi/HUAP	1992	Inv	CG	III	Vc	III	B	A_1
BMB6792	Niteroi/HUAP	1992	Inv	CG	III	Vc	III	B	A_1
BMB7192	Niteroi/HUAP	1992	Inv	CG	III	Vc	III	B	A_1
BMB7292	Niteroi/HUAP	1992	Inv	CG	III	Vc	III	B	A_1
BMB17992	Niteroi/HUAP	1992	Inv	CG	III	Vc	III	B	A_1
BMB11392	Sao Paulo/HSSP	1992	Inv	CG	III	Vc	III	B	A_1
BMB11492	Sao Paulo/HSSP	1992	Inv	CG	III	Vc	III	B	D
BMB11892	Sao Paulo/HSSP	1992	Inv	CG	III	Vc	III	B	A_1
BMB12892	Sao Paulo/HSSP	1992	Inv	CG	III	Vc	III	ND	A_1
BMB13192	Sao Paulo/HSSP	1992	Inv	CG	III	Vc	III	B	F
BMB13392	Sao Paulo/HSSP	1992	Inv	CG	III	Vc	III	ND	G
BMB13592	Sao Paulo/HSSP	1992	Inv	CG	III	Vc	III	ND	A_6
BMB13792	Sao Paulo/HSSP	1992	Inv	CG	III	Vc, Cip, Tc, SfT, Cl	II	ND	B
BMB13892	Sao Paulo/HSSP	1992	Inv	CG	III	Vc, Cip, Tc, SfT, Cl	II	ND	B
BMB13992	Sao Paulo/HSSP	1992	Inv	CG	III	Vc	III	ND	A_1
BMB14092	Sao Paulo/HSSP	1992	Inv	CG	III	Vc	III	ND	A_6

[a] MRSA strains were isolated from the following hospitals: Hospital de Clinicas de Porto Alegre (HCPA), Hospital da Universidade Federal de Manaus (HUFM), Hospital Universitario Clementino Fraga Filho (HUCFF), Hospital Samaritano (HS), Hospital Universitario Antonio Pedro (HUAP), and Hospital dos Servidores do Estado de Sao Paulo (HSSP).

[b] Inv, invasive infection; the origin of the cultured material is not recorded; C, colonizing strains obtained from noses of patients; HCP, colonizing strains obtained from noses of health personnel.

[c] Agar screen with 25 μg of methicillin per ml (4). CG, confluent growth on methicillin agar.

[d] PAP, population analysis profiles.

[e] All strains were resistant to penicillin, oxacillin, cephalothin, erythromycin, and gentamicin, and many strains were also resistant to chloramphenicol (Co), clindamycin (Cl), tetracycline (Tc), ciprofloxacin (Cip), and trimethoprim-sulfamethoxazole (SfT). The antibiotics to which the isolates remained susceptible are indicated. All strains remained susceptible to vancomycin (Vc).

[f] *mecA* polymorph, as defined according to Kreiswirth et al. (9).

[g] Strains assigned the same letter but with a different subindex represent minor variants (less than a four-band difference) with the same chromosomal background.

[h] ND, not done.

pattern A (Fig. 1A). Every one of these 66 isolates carried the same *mecA* polymorph, polymorph III (9), and each of the strains examined in this group of 39 strains also had a common Tn*554* pattern, pattern B (Fig. 2A and B). Isolates belonging to this *mecA* III::Tn*554* B:PFGE A clone represented the majority of MRSA isolates at each of the hospital centers. The *mecA* polymorph III was also detected in combination with four additional, minor PFGE types (D, F, G, and H; Table 1). *mecA* polymorphs I and II were detected in only one and four strains, respectively.

PFGE analysis of 38 methicillin-susceptible or borderline resistant *S. aureus* isolates (collected at the three hospitals in the state of Rio de Janeiro and from Porto Alegre) showed a large variety of distinct PFGE patterns (17 patterns in 38 isolates) (Fig. 1B), similar to a finding in a Spanish hospital described previously (5).

All 85 MRSA isolates showed a common methicillin resistance phenotype (class 3), and most strains (>70%) were multiresistant: they carried traits of resistance to 9 additional antibiotics.

The observations described here identify a multiresistant MRSA clone with a high level of methicillin resistance and wide geographic spread in the south, southeastern, and northern regions of Brazil. The two hospitals in Rio de Janeiro and Porto Alegre are located approximately 1,600 km from each other, and Manaus is an additional 3,700 km north of Rio de Janeiro. Therefore, it is likely that the spread of these strains occurred indirectly via the community rather than through the sharing of patients or staff (6).

TABLE 2. Antimicrobial resistance patterns of methicillin-susceptible, borderline methicillin-resistant, and methicillin-resistant isolates of *S. aureus* from Brazilian hospitals

Antimicrobial resistance pattern[a]	% Distribution of *S. aureus*[b]		
	Susceptible	Borderline resistant	MRSA (phenotype)[c]
None	13	9	
Pn	52	62	
Tc	7		
Pn, Er	7	9	
Pn, Tc	7	11	
Pn, SfT	7		
Pn, Gm		5	
Pn, Co		2	
Pn, Er, Tc	7		
Pn, Er, Gm, Co		2	
Pn, Er, Gm, Co, Cf			3 (3)
Pn, Er, Gm, Co, Cf, Tc, Cl			1 (3)
Pn, Er, Gm, Cf, Tc, SfT, Cip, Cl			3 (3)
Pn, Er, Gm, Co, Cf, Tc, SfT, Cip			6 (3)
Pn, Er, Gm, Co, Cf, Tc, Cip, Cl			5 (3)
Pn, Er, Gm, Co, Cf, Tc, SfT, Cl			8 (3)
Pn, Er, Gm, Co, Cf, Tc, SfT, Cip, Cl			74 (3)

[a] The following antimicrobial disks were used: penicillin (Pn; 10 U), erythromycin (Er; 15 μg/ml), gentamicin (Gm; 10 μg/ml), chloramphenicol (Co; 30 μg/ml), cephalothin (Cf; 30 μg/ml), tetracycline (Tc; 30 μg/ml), trimethoprim-sulfamethoxazole (SfT; 1:19; 25 μg/ml), ciprofloxacin (Cip; 5 μg/ml), clindamycin (Cl; 2 μg/ml), and vancomycin (Vc; 30 μg/ml). The tests were carried out as recommended by the National Committee for Clinical Laboratory Standards (12).

[b] The *S. aureus* isolates were grouped on the basis of agar screen, DNA probe, and population analysis profiles into methicillin-susceptible, borderline resistant, and MRSA strains. For strains classified as borderline resistant, methicillin MICs were 2 to 4 μg/ml, and these strains did not give a positive signal when they were tested with the *mecA* DNA probe (4, 12).

[c] The methicillin resistance phenotype class was determined by population analysis profiles.

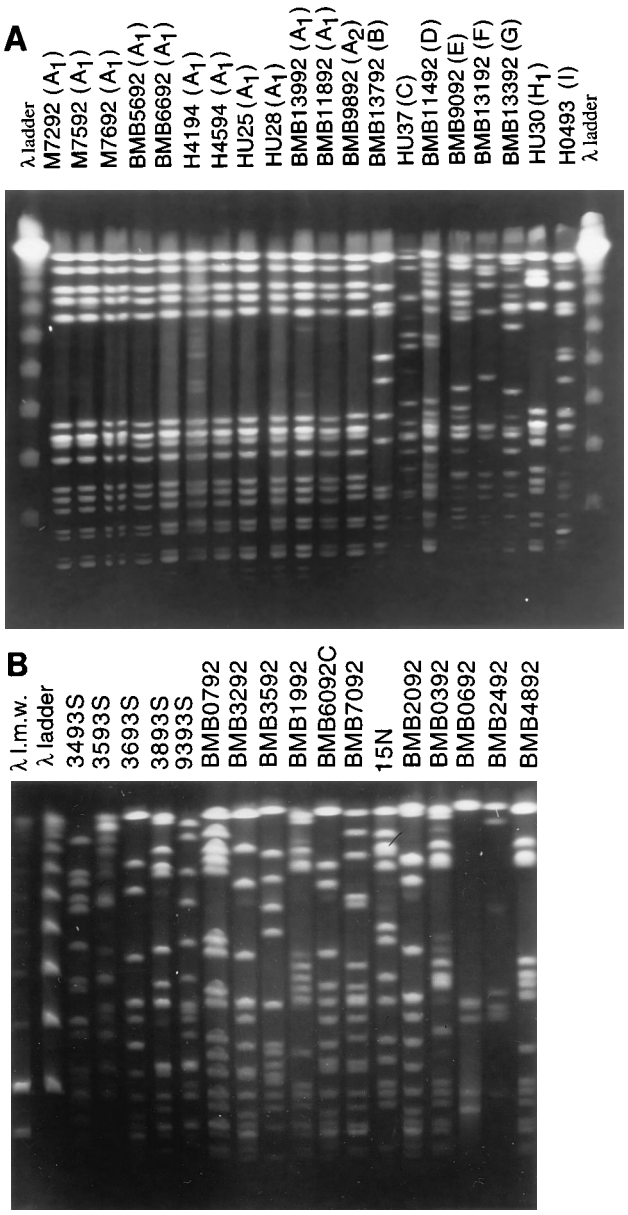

FIG. 1. PFGE patterns of multidrug-resistant MRSA isolates (the PFGE type is indicated by the letter in parentheses) (A) and methicillin-susceptible and/or borderline-resistant *S. aureus* isolates (B) from several Brazilian hospitals. The origins of the isolates are indicated by the notations above the lanes (Table 1).

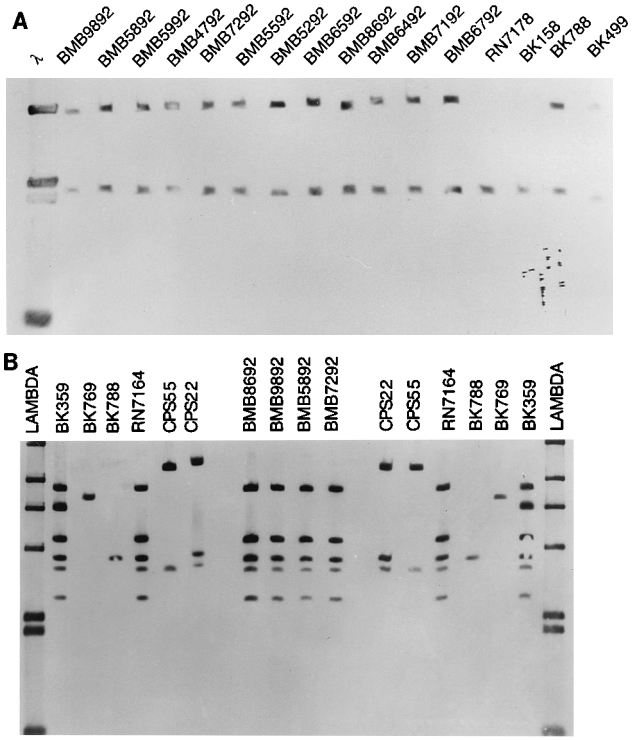

FIG. 2. *mecA* polymorphs and Tn*554* patterns associated with the Brazilian MRSA isolates. Chromosomal DNAs were restricted with the *Cla*I endonuclease, and the electrophoretically separated fragments were hybridized by a *mecA*-specific DNA probe (A) as described in Materials and Methods. After removal of the radioactive probe, the same membranes were subsequently hybridized with the Tn*554* probe (B). The origins and some relevant properties of the strains marked BMB are described in Table 1. (A) Lanes RN7178, BK158, BK788, and BK499, reference strains for *mecA* polymorphs (9). (B) The four center lanes marked BMB illustrate the dominant Tn*554* pattern of the Brazilian MRSA isolates. The remaining lanes contain six reference strains of Tn*554* patterns associated with the *mecA* polymorph III (3, 9).

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